

# Photochemical and Thermal Bergman Cyclization of a Pyrimidine Eneidiynol and Eneidiynone

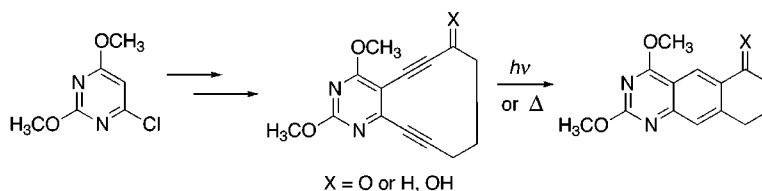
Nakyen Choy,<sup>†</sup> Beatriz Blanco,<sup>†</sup> Jenghai Wen,<sup>‡</sup> Awtar Krishan,<sup>‡</sup> and K. C. Russell<sup>\*†</sup>

Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, Florida 33124, and Department Of Radiation Oncology (R-71), University of Miami, P.O. Box 016960, Miami, Florida 33101

krussell@umiami.ir.miami.edu

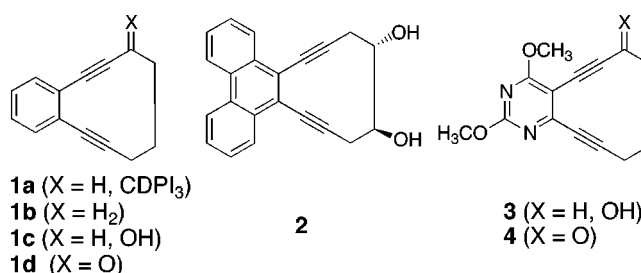
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## ABSTRACT



Novel 10-membered pyrimidine enediynes (**3** and **4**) were synthesized in seven and eight steps, respectively. These compounds were compared for their abilities to undergo Bergman cyclization both thermally and photochemically. Alcohol **3** readily cyclized both thermally and photochemically in <sup>1</sup>PrOH, while ketone **4** only showed efficient thermal cyclization. Both compounds were also shown to cleave dsDNA under the appropriate conditions.

Since the natural enediyne anticancer antibiotics<sup>1</sup> were first reported, designed enediynes have been synthesized and studied as DNA cleaving agents on the basis of the Bergman cyclization.<sup>2</sup> Synthetic enediynes have a distinct advantage over the naturally occurring products as therapeutic agents because they may show reduced toxicity.<sup>3</sup> The parameters for thermal enediyne activation by modification of 10-membered rings<sup>4</sup> such as **1** have been well studied. Introduction of an alcohol (**1c**) adjacent to the triple bond tends to accelerate reactivity relative to that of **1b**. The ketone (**1d**) further activates cyclization by increasing the ring strain from



the change in hybridization. It is also likely there is an electronic effect from the ketone, decreasing the electron density available to the enediyne. However, there are relatively few examples of photochemical Bergman cyclizations<sup>5</sup> (e.g., **2<sup>5c</sup>**). Despite the potential of the photo-Bergman cyclization in photodynamic therapy, little has been done to

\* Phone (305)284-6856. Fax (305)284-1880.

<sup>†</sup> Department of Chemistry.

<sup>‡</sup> Department of Radiation Oncology.

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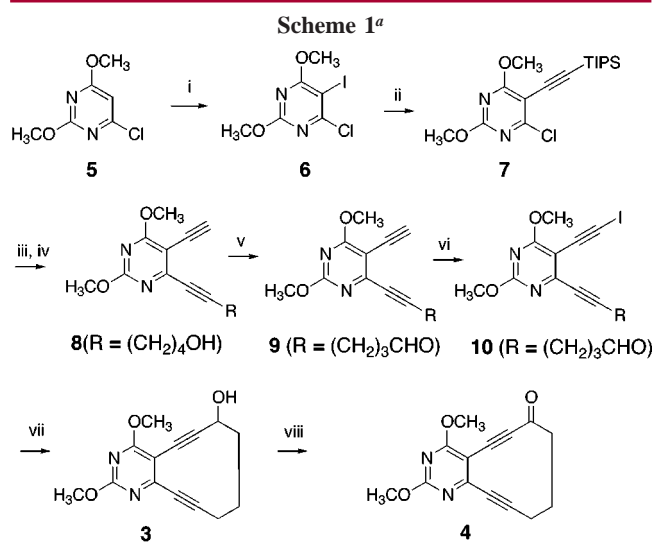
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explore how substitution on the 10-membered ring affects photoinduced cyclization. Our group has been interested in the examination of enediyne compounds where the double bond of the enediyne has been incorporated into biologically relevant heterocycles.<sup>6</sup> In this Letter we present the difference in the reactivity of pyrimidine enediyne **3** and enediyne **4** toward photochemical Bergman cyclization and contrast that with results from thermal cyclization under similar conditions. In addition, the DNA cleavage abilities of these new enedynes under thermal and photochemical conditions are reported.

The syntheses of enedynes **3** and **4** are depicted in Scheme 1. Compound **6** was prepared according to our previous



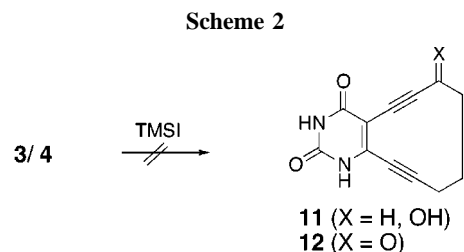
<sup>a</sup> Reagents: i,  $(\text{CF}_3\text{CO})_2\text{O}-\text{CF}_3\text{CO}_2\text{H}$  (1:4), NIS (1.5 equiv), reflux, 10 h, 95%; ii, TIPS-C≡C-H (1.2 equiv), Pd(PPh<sub>3</sub>)<sub>4</sub> (10% w/w), CuI (1 equiv), <sup>i</sup>Pr<sub>2</sub>NH-THF (1:1), 70 °C, 10 h, 85%; iii, H-C≡C(CH<sub>2</sub>)<sub>4</sub>OH, Pd(PPh<sub>3</sub>)<sub>4</sub>, <sup>i</sup>Pr<sub>2</sub>NH, reflux, 15 h; iv, TBAF, THF, 0 °C, 0.5 h, 65% (two steps); v, PCC (3 equiv), CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h, 93%; vi, NIS (1.2 equiv), AgNO<sub>3</sub> (0.3 equiv), acetone, rt, 1 h, 91%; vii, CrCl<sub>2</sub> (10 equiv), NiCl<sub>2</sub> (0.1 equiv), THF, rt, 65%; viii, MnO<sub>2</sub> (10 equiv), CH<sub>2</sub>Cl<sub>2</sub>, rt, 5 h, 86%.

work.<sup>6c</sup> Coupling **6** with TIPS-protected acetylene using CuI and Pd(PPh<sub>3</sub>)<sub>4</sub> as a catalyst in a mixture of diisopropylamine-THF at 70 °C under N<sub>2</sub> gave monocoupled product **7** and a trace of the dicoupled product (0.4% by GC). This crude product was coupled with 5-hexyn-1-ol using catalytic Pd(PPh<sub>3</sub>)<sub>4</sub> in diisopropylamine followed by removal of the silyl group with tetrabutylammonium fluoride, affording **8** in good yield. The corresponding aldehyde **9** was prepared by PCC oxidation. Iodination of the terminal acetylene using

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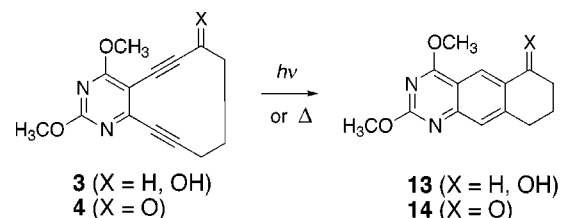
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NIS and silver nitrate<sup>7</sup> gave **10** in excellent yield. Iodination of **8** or **9** using I<sub>2</sub> and a base (morpholine,<sup>4a</sup> DBU,<sup>8a</sup> or DMAP<sup>8b</sup>) provided the product in low yield (10–20%). The key ring closure of **10** was carried out with a CrCl<sub>2</sub>-NiCl<sub>2</sub> system.<sup>9</sup> Oxidation of **3**<sup>10</sup> with MnO<sub>2</sub> was used to prepare **4**.<sup>11</sup> Several unsuccessful attempts were also made to prepare pyrimidinone analogues **11** and **12** by TMSI cleavage of the methyl lactim ethers (Scheme 2).<sup>6b</sup>



Alcohol **3** underwent both thermal and photochemical cyclization in isopropyl alcohol as a solvent as well as radical acceptor in excellent yield (Table 1, entries 1 and 2). Under

**Table 1.** Thermal and Photochemical Bergman Cyclization of **3** and **4**



entry	compd	solvent (time, h)	[concn] of <b>3</b> or <b>4</b>	temp, condn	<b>13/14</b> yield (%)
1	<b>3</b>	<sup>i</sup> PrOH (10)	0.020	reflux, Δ	93 <sup>a</sup>
2	<b>3</b>	<sup>i</sup> PrOH (24)	0.001	40 °C, <i>hν</i> <sup>c</sup>	82 <sup>a</sup>
3	<b>3</b>	<sup>i</sup> PrOH (36)	0.001	40 °C, Δ	trace <sup>b</sup>
4	<b>3</b>	<sup>i</sup> PrOH (2)	0.005	40 °C, <i>hν</i> <sup>d</sup>	(4 <sup>g</sup> )83 <sup>f</sup>
5	<b>4</b>	<sup>i</sup> PrOH (10)	0.020	80 °C, Δ	92 <sup>a</sup>
6	<b>4</b>	<sup>i</sup> PrOH (2)	0.005	40 °C, <i>hν</i> <sup>d</sup>	(34 <sup>g</sup> )10 <sup>f</sup>

<sup>a</sup> Isolation yield. <sup>b</sup> By <sup>1</sup>H NMR. <sup>c</sup> Filter solution, [K<sub>2</sub>CrO<sub>4</sub>] = 0.01 M. <sup>d</sup> Filter solution, [K<sub>2</sub>CrO<sub>4</sub>] = 0.001 M. <sup>e</sup> Remaining starting material. <sup>f</sup> By HPLC with internal standard, xylene.

photochemical conditions<sup>12</sup> using a filter solution (aqueous K<sub>2</sub>CrO<sub>4</sub>), **3** cyclized in good yield at 40 °C (entry 2), yet it cyclized extremely slowly thermally at the same temperature (entry 3). Under photochemical conditions at 40 °C, the half-

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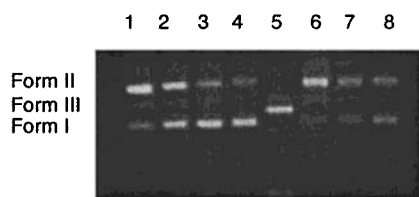
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life was 29 min ( $[3] = 0.005$  M in  $i$ PrOH,  $[K_2CrO_4] = 0.001$  M, correlation = 0.997), while the thermal cyclization half-life at 60 °C was 58 h ( $[3] = 0.005$  M in  $i$ PrOH, correlation = 0.995).

Ketone **4** underwent thermal cyclization in isopropyl alcohol in excellent yield (entry 5). Under thermal conditions at 40 °C, the half-life was 55 h ( $[4] = 0.005$  M in  $i$ PrOH, correlation = 0.996). However, compound **4** did not undergo efficient photochemical cyclization under conditions identical to those of **3**. After a 2 h irradiation, only 10% of the cyclized product was observed (entry 6), unlike **3** which showed 83% of the product (entry 4). The reaction also showed a poor mass balance with only 34% of starting material remaining. We determined that the poor yields of cyclization product stem from photochemical processes involving enediyne **4** and not from decomposition of **14** after cyclization. This was accomplished by irradiating **14** (isolated from thermal cyclization) under the same photochemical conditions as used in the cyclization of **3** and **4**. The experiment clearly demonstrated that ketone **14** was stable over the time scale that led to poor cyclization of **4** and significant side products (entry 6).

The difference in reactivity between alcohol **3** and ketone **4** may arise from different excited states. Ketones are well-known to possess excited states and reactivity different from olefins and aromatics. Ketones readily form triplet excited states which can undergo hydrogen and electron abstraction processes.<sup>13</sup> If the photochemical Bergman cyclization is favored by a singlet excited state, then a triplet state ketone could interfere with the normal cyclization process and result in the poor yields and conversion observed for **4**.

Compounds **3** and **4** were also examined for their ability to cleave double-stranded DNA. Figure 1 shows the results



**Figure 1.** Supercoiled DNA interaction  $\Phi$ X174. DNA was incubated for 70 h at 40 °C with compounds **3** and **4** in buffer (TE, pH 7.6) and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lanes 1–3: **3** (4000, 400, and 40  $\mu$ M). Lane 4: DNA control. Lane 5: DNA control with restriction enzyme, Dra I. Lanes 6–8: **4** (4000, 400, and 40  $\mu$ M).

obtained after incubating **3** and **4** with  $\Phi$ X174 dsDNA for 70 h at 40 °C. The ketone shows significant DNA nicking (Form II) at concentrations as low as 40  $\mu$ M and nearly

(10) **3**: mp 108–110 °C; UV ( $i$ PrOH)  $\lambda_{max}$  ( $\epsilon$ ) 311 nm (7100), 260 nm (9200), 249 nm (10300); FT-IR ( $CH_2Cl_2$ ) 3590, 2352, 1572, 1525, 1378  $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  4.68 (m, 1H), 4.02 (s, 3H), 3.98 (s, 3H); 2.52 (m, 2H), 2.29–1.84 (m, 5H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  168.9, 163.9, 158.8, 107.6, 104.0, 103.3, 80.2, 79.1, 63.1, 55.3, 54.6, 37.5, 23.0, 21.3; HRMS (DEI) calcd for  $C_{14}H_{14}N_2O_3$  258.1004, found 258.0994.

complete nicking at 4 mM. The alcohol showed almost no reactivity at 40  $\mu$ M but was able to nick DNA at 4 mM. While no double-strand (ds) cleavage (Form III) was observed at the lower concentrations, both **3** and **4** did show slight ds cleavage at 4 mM. The thermal cleavage properties of **3** are similar to those of alcohol **1c**.<sup>4b</sup>

Likewise, photochemical DNA cleavage was demonstrated for **3** and **4** ( $h\nu$ , 40 °C, 3 h; gel not shown). In this case, compound **3** showed superior DNA cleavage ability. At 40  $\mu$ M, compound **3** showed significant DNA single-strand cleavage while compound **4** showed no discernible activity. At higher concentrations (4000  $\mu$ M), both compounds showed signs of double-strand scission, again with **3** giving the more complete reaction. The photochemical DNA cleavage properties of **4** compare favorably with *O*-alkylated derivatives of pyrene **2**.<sup>5c</sup>

Compounds **3** and **4** were also examined for their anticancer activity. Human leukemic lymphoblasts of the CCRF-CEM cell line (log-phase cultures) were incubated with 2–40  $\mu$ M of **3** or **4** for 24 h to test the effect on cell viability and cell cycle traverse. After staining with propidium iodide/hypotonic citrate,<sup>14</sup> aliquots were analyzed by laser flow cytometry. In cultures exposed to 2  $\mu$ M of alcohol **3**, growth inhibition and cytotoxicity were indicated by the reduction in the number of cells with S and  $G_2/M$  DNA content accompanied by the appearance of cells with less  $G_0/G_1$  DNA content (apoptotic cells?). At higher concentrations (4  $\mu$ M) alcohol **3** caused accumulation of cells in  $G_0/G_1$ -early S-phase accompanied by a significant increase in the number of apoptotic cells. Ketone **4** at 2  $\mu$ M did not have any significant effects on cell cycle traverse, but in cultures exposed at 4  $\mu$ M, there was a pronounced reduction in the number of cells with the  $G_0/G_1$  DNA content accompanied by an accumulation of cells with late-S and  $G_2/M$  DNA content. The number of cells with  $G_0/G_1$  DNA content also increased. Alcohol **3** and ketone **4** both had  $IC_{50}$  values of  $\approx 1.25$   $\mu$ M.

In conclusion, the first cyclic pyrimidine enediynes (**3** and **4**) were synthesized with 29% and 25% overall yields in seven and eight steps, respectively. Compound **3** cyclized with a half-life of 29 min at 40 °C under the photochemical conditions used here. The ketone does not efficiently cyclize under the same photochemical conditions. Both the ketone and the alcohol were able to effect DNA cleavage at reasonable concentrations at physiological temperatures. As

(11) **4**: mp 110 °C (decomp); UV ( $i$ PrOH)  $\lambda_{max}$  ( $\epsilon$ ) 323 nm (16100), 286 nm (14100), 249 nm (37000); FT-IR ( $CH_2Cl_2$ ) 2257, 2173, 1793, 1656, 1567, 1520, 1473, 1378  $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  4.00 (s, 3H), 3.99 (s, 3H), 2.84 (t, 2H,  $J = 5.6$  Hz), 2.63 (t, 2H,  $J = 5.6$  Hz), 2.17 (m, 2H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  186.7, 169.9, 165.4, 161.1, 107.8, 101.6, 99.0, 90.6, 80.2, 55.6, 54.8, 46.3, 24.8, 22.1; HRMS (DEI) calcd for  $C_{14}H_{12}N_2O_3$  256.0848, found 256.0839.

(12) **General procedure for the photochemical reaction**: Photolysis experiments were carried out with a Rayonette photochemical reactor equipped with 16 3100 Å lamps, and a 0.01 M potassium chromate ( $K_2CrO_4$ ) filter solution was employed to filter out the 313 nm wavelength. A solution of **3** (2.58 mg, 0.01 mmol) in degassed  $i$ PrOH (10 mL) was stirred at 40 °C for 24 h. After the solvent was evaporated under reduced pressure, the reaction mixture was purified by column chromatography ( $SiO_2$ , hexane/ethyl acetate, 75/25), from which pure **13** was obtained (82%).

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expected from the cyclization studies, **3** gave better DNA cleavage under photochemical conditions while **4** was superior under thermal conditions alone. Flow cytometric studies showed that while both compounds caused an increase in the number of cells with less G<sub>0</sub>/G<sub>1</sub> DNA content (possibly apoptotic cells), their effects on cell cycle traverse were different. While alcohol **3** caused accumulation of cells in G<sub>0</sub>/G<sub>1</sub>-early S-phase of the cell cycle, ketone **4** caused accumulation of cells in the G<sub>2</sub>/M part of the cell cycle. The presence of apoptotic cells in cultures exposed to these compounds may suggest the involvement of the apoptotic mechanism in cytotoxicity while the presence of cells with G<sub>2</sub>/M DNA content may indicate interference with DNA synthesis and repair mechanisms. The involvement of enediynes in apoptosis through specific receptor–ligand interactions has been previously proposed.<sup>15</sup>

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Investigations including detailed kinetic experiments on **3** and **4** and the synthesis of pyrimidinone analogues **11** and **12** are in progress.

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